Cell biology

Assembly surprise for membrane proteins

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Membrane-spanning proteins have many vital roles in the cell. New findings challenge current understanding of the route by which such proteins are inserted into the membrane of the endoplasmic reticulum.
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Proteins in cellular membranes contain one or more membrane-spanning segments, called transmembrane domains (TMDs). Insertion of these TMDs into the membrane is a key step in the production of new membrane proteins. In eukaryotic cells (those with a nucleus) this insertion process occurs mainly at one organelle — the endoplasmic reticulum. Here, TMDs are integrated into the membrane as they emerge from the protein-producing ribosome. For many decades the accepted view has been that TMD insertion occurs through the same machinery - the Sec61 complex - that transports proteins into the interior of the endoplasmic reticulum[1,2]. However, writing in *Nature*, Smalinskaite *et al.*[3] and Sundaram *et al.*[4] challenge this understanding.

Smalinskaite *et al.*[3] and Sundaram *et al.*[4] show that when ribosomes are inserting proteins with multiple TMDs (termed multipass proteins) they recruit not only the Sec61 complex but also additional membrane proteins to form what the authors call the Multipass Translocon (MPT). Moreover, the authors report the remarkable finding that some multipass proteins which trigger assembly of the MPT are inserted into the membrane without passing through the classic Sec61 complex at all. These studies overturn the current paradigm that TMDs are inserted into the membrane exclusively by the Sec61 complex and instead paint a picture in which responsibility for TMD integration is passed to the MPT as protein synthesis proceeds.

A role for the Sec61 complex in membrane protein insertion is well-established. However, the authors of the current studies had previously identified additional endoplasmic reticulum membrane proteins that seemed to be associated with the generation of multipass membrane proteins[5,6]. The new studies now confirm that these additional proteins are part of a linked network of three ribosome-bound complexes termed the GEL, PAT and BOS complexes. These complexes are positioned in the membrane beside the Sec61 complex to form the MPT (Fig. 1). Deletion of MPT components impairs the correct insertion of multipass membrane proteins, but not membrane proteins with a single TMD, confirming the involvement of these MPT components in multipass protein formation[4,6] Intriguingly, the MPT is not the only new player in multipass membrane protein biogenesis. Another recently-discovered endoplasmic reticulum complex called EMC inserts the first TMD of certain proteins into the membrane prior to the ribosome docking with Sec61[7].

Smalinskaite *et al.* and Sundaram *et al.* investigate the time course of multipass protein insertion through characterizing intermediates stalled at different stages during the integration process. They find that ribosomes synthesizing multipass membrane proteins initially interact with the established insertion machinery comprised of the Sec61 complex, a complex of poorly-defined function called TRAP, and the oligosaccharyl transferase complex (OST) that adds sugar groups to most inserted proteins (Fig.1). However, once an initial segment of the protein containing from one to three TMDs has been integrated into the membrane, the MPT is recruited to the ribosome and this association is retained during the insertion of further TMDs. The MPT occupies the same space underneath the ribosome as OST. Consequently, MPT recruitment requires that OST first be displaced from the insertion apparatus. The remodelling of the ribosome-associated insertion apparatus appears to be

triggered by features within the multipass protein that is being synthesized. An obvious challenge for future research is to elucidate exactly how the protein being inserted into the membrane drives these changes.

A key additional finding from these studies is that the Sec61 complex is no longer involved in the integration of TMDs once the MPT has assembled. The first evidence for this conclusion is structural. The Sec61 complex forms a transmembrane channel structure that enables it to carry out the dual functions of protein transport and TMD integration. Water-soluble proteins cross the membrane through the channel, whereas TMDs exit the channel into the membrane through opening a side seam of the complex termed the lateral gate[8] (Fig. 1). Smalinskaite *et al.*[3] show that recruitment of the MPT locks the lateral gate of the Sec61 complex closed and thus blocks TMD insertion through this route. The second piece of evidence for a bypass of the Sec61 complex comes from experiments in which Smalinskaite *et al.*[3] examine the membrane insertion of MPT-recruiting multipass proteins in which the first TMD is inserted by EMC rather than by Sec61[7]. They find that membrane insertion of these proteins is unaffected by inhibitors of the Sec61 complex. Thus, there are MPT-recruiting multipass proteins that can be routed into the membrane without passing through the Sec61 complex at any stage. Considered together these observations suggest that the MPT takes over the role of TMD insertion from Sec61 (or in some cases from EMC) in the later stages of the formation of multipass membrane proteins.

The observations of Smalinskaite *et al.* and Sundaram *et al.* lead to a revised model of multipass protein generation (Fig. 1). In this model the ribosome is initially targeted to a complex containing Sec61 and OST, consistent with the current model for membrane protein formation. However, only the first few TMDs of the substrate protein are integrated into the membrane by the Sec61 complex. Instead, at this point OST disengages from the ribosome and is replaced by the MPT which takes over the insertion of the membrane protein from the Sec61 complex. For those multipass proteins in which the first TMD is integrated by EMC, the Sec61 complex may not be involved in TMD integration at all but instead serves to dock the ribosome to the membrane to allow subsequent MPT assembly.

The roles the various components of the MPT play in the membrane protein insertion process and how their interactions with the inserting protein are choreographed remain to be determined. The PAT complex probably sequesters any polar regions in the TMDs away from the non-polar membrane environment until they can be buried in the interior of the fully assembled protein[6]. One subunit of the GEL complex is similar to a bacterial protein that can have TMD-inserting activity[3,5], and therefore is a good candidate to 'feed' TMDs into the membrane. It is also possible that some of the TMDs might directly integrate into the membrane without requiring assistance from other proteins because the structure of one of the integration intermediates reported by Smalinskaite *et al.* shows that the most recently integrated TMD is located in the membrane between the Sec61 and MPT complexes. Not all components of the MPT have obvious equivalents in non-animal eukaryotes raising the question of why mammalian multipass proteins require a more complex biosynthetic apparatus than that of other eukaryotes.

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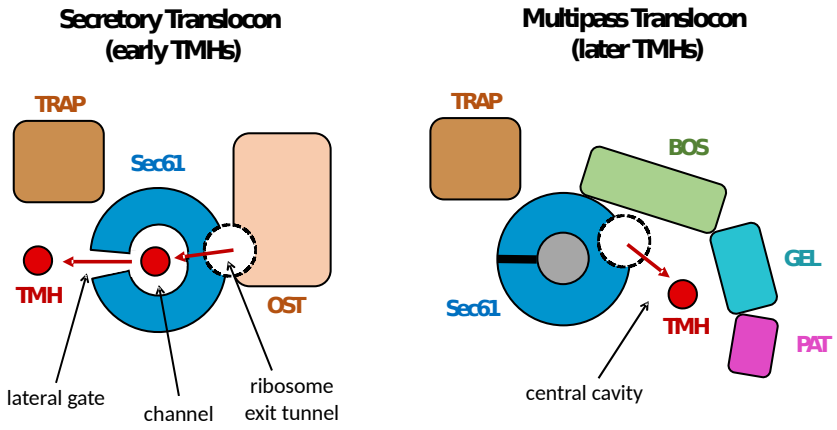

Figure 1. A model for the insertion of multipass membrane proteins into the mammalian endoplasmic reticulum based on the findings of Smalinskaite *et al.*[3] and Sundaram *et al.*[4] The insertion machinery within the membrane is shown in schematic representation viewed from the cytoplasm. The approximate position of the ribosome exit tunnel above this machinery is indicated. Ribosomes synthesizing multipass membrane proteins are initially targeted to the Secretory Translocon (Left) where the initial transmembrane domains (TMDs) are routed into the membrane via the channel and lateral gate of the Sec61 complex (for some proteins the initial TMD has already been inserted by alternative mechanism involving a protein called EMC and the Sec61 complex is only used to dock the ribosome to the membrane). Once the initial portion of the protein has entered the membrane, the insertion complex is remodelled to form the Multipass Translocon by displacement of the oligosaccharyl transferase (OST) complex and recruitment of the BOS, GEL, and PAT complexes (Right). In the Multipass Translocon entry into the channel of Sec61 is occluded and the lateral gate is locked shut. TMDs exiting the ribosome are now directed towards the Multipass Translocon complexes which inserts them into the membrane of the central cavity. TRAP is a Sec61-associated complex of poorly defined function.

[1] Rapoport T.A., Li L. & Park E. *Annu Rev Cell Dev Biol* **33**, 369-390 (2017).
doi: 10.1146/annurev-cellbio-100616-060439.
[2] Hegde R.S. & Keenan R.J. *Nat Rev Mol Cell Biol* **23**,107-124 (2022).
doi: 10.1038/s41580-021-00413-2.
[3] Smalinskaite paper.
[4] Sundaram paper.
[5] McGilvray P.T. *et al. Elife* **9**, e56889 (2020).
doi: 10.7554/eLife.56889.
[6] Chitwood P.J. & Hegde R.S. *Nature* **584**, 630-634 (2020).
doi: 10.1038/s41586-020-2624-y.
[7] Chitwood P.J., Juszkiewicz S., Guna A., Shao S. & Hegde R.S. *Cell* **175**, 1507-1519.e16. (2018)
doi: 10.1016/j.cell.2018.10.009.
[8] Van den Berg B. *et al. Nature* **427**, 36-44 (2004).
doi: 10.1038/nature02218.